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A DNA Integrity Network in the Yeast *Saccharomyces cerevisiae*

Xuewen Pan,[1,2] Ping Ye,[2,3] Daniel S. Yuan,[1,2] Xiaoling Wang,[1,2] Joel S. Bader,[2,3] and Jef D. Boeke[1,2,*]
[1] Department of Molecular Biology and Genetics
[2] The High Throughput Biology Center
The Johns Hopkins University School of Medicine, 733 North Broadway, Baltimore, MD 21205, USA
[3] Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218 USA
*Contact: jboeke@jhmi.edu
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SUMMARY

A network governing DNA integrity was identified in yeast by a global genetic analysis of synthetic fitness or lethality defect (SFL) interactions. Within this network, 16 functional modules or minipathways were defined based on patterns of global SFL interactions. Modules or genes involved in DNA replication, DNA-replication checkpoint (DRC) signaling, and oxidative stress response were identified as the major guardians against lethal spontaneous DNA damage, efficient repair of which requires the functions of the DNA-damage checkpoint signaling and multiple DNA-repair pathways. This genome-wide genetic interaction network also identified novel components (*DIA2*, *NPT1*, *HST3*, *HST4*, and the *CSM1* module) that potentially contribute to mitotic DNA replication and genomic stability and revealed novel functions of well-studied genes (the *CTF18* module) in DRC signaling. This network will guide more detailed characterization of mechanisms governing DNA integrity in yeast and other organisms.

INTRODUCTION

Permanent genetic change contributes to the onset of human diseases and aging. In particular, genomic instability is a driving force for cancer development (Lengauer et al., 1998). All organisms are constantly exposed to exogenous and endogenous genotoxic assaults that challenge genome integrity. Cells are normally armed with various mechanisms that minimize, detect, and repair DNA lesions to preserve genome integrity. These mechanisms are well understood in the model organism *Saccharomyces cerevisiae* and are mostly shared by higher organisms including humans (Elledge, 1996; Rouse and Jackson, 2002). Preservation of genomic integrity involves multiple biological processes, including DNA replication, DNA repair, and signaling pathways that coordinate DNA metabolism with cell cycle progression (Kolodner et al., 2002). However, a global view of the interrelationships among these processes is still lacking. Moreover, new components remain uncovered and new functions of known molecules remain to be revealed. We describe the use of synthetic lethality to provide a global view of the DNA integrity network.

Synthetic lethality defines a genetic relationship between a pair of alleles, where either allele on its own allows cell survival but the combination of both prevents or severely retards growth. Synthetic lethality can occur between genes acting in the same biochemical pathway or in distinct but compensatory pathways (Hartman et al., 2001). Here, we exploited this phenomenon to probe the functional relationships among different processes contributing to DNA integrity in yeast. We used the dSLAM approach (diploid synthetic lethal analysis by microarray; Pan et al., 2004) and the yeast knockout (YKO) heterozygous mutant collection (Giaever et al., 2002) to conduct synthetic lethality analyses on a genome-wide scale. Starting with 74 query genes, 4956 unique pairs of synthetically fitness or lethal defect (SFL) interactions involving 875 genes were uncovered. Importantly, only ~9% of these interactions were previously reported (Huang and Kolodner, 2005; Tong et al., 2004).

With these SFL interactions, a number of functionally connected multicomponent modules or minipathways were defined. Extensive functional compensation was observed among distinct processes contributing to DNA integrity in yeast. These include DNA replication, oxidative stress response, DNA repair, checkpoint signaling, and chromatin structure maintenance. The interactions identified also suggest specific roles for mRNA transcription, mRNA processing, and Golgi functions in maintaining DNA integrity. Combining mutations in compensatory pathways or processes likely leads directly to increased endogenous DNA damage and genomic instability. New genes and pathways implicated in DNA replication and other pathways were also identified by these analyses. These include *DIA2*, which encodes an F box protein required for ubiquitin-dependent protein degradation (Bao et al., 2004), *NPT1*, the gene of a nicotinate phosphoribosyltransferase required for biosynthesis of NAD^+ via the salvage pathway (Rajavel et al., 1998), and genes of the

Sir2p homologs Hst3p and Hst4p (Brachmann et al., 1995). In particular, Dia2p may regulate the turnover of Ctf4p, abundant expression of which is detrimental to yeast cells. Additionally, we have discovered new functions for some well-studied proteins. The Ctf18p/Ctf8p/Dcc1p complex, required for sister chromatid cohesion (Mayer et al., 2001) also plays an important role in DNA-replication checkpoint signaling. The Rad53p protein kinase might play a direct role in chromatin-structure maintenance. This data set is likely to generate numerous further molecular inroads to more detailed characterization of the mechanisms governing DNA integrity in yeast and other organisms.

RESULTS

Distinct Functional Modules Contributing to DNA Integrity

Most biological functions are carried out by multicomponent pathways and not by isolated individual proteins. Thus, to study a biological network, it is important to first identify the pathways that carry out each biological function. Components of the same pathway tend to share similar synthetic lethality partners (Ye et al., 2005; Tong et al., 2004), and synthetic lethality interactions normally do not occur between pairs of null mutations of the same linear pathway (Kelley and Ideker, 2005; Ye et al., 2005). We use the term congruence to mathematically define the similarity in SFL interaction lists (Ye et al., 2005).

In this study, we have identified 4956 unique SFL interactions with 74 queries; the majority of these interactions are relevant to DNA integrity maintenance (see Table S1 in the Supplemental Data available with this article online). With this data set, we first attempted to identify functionally distinct modules or linear pathways. We required that components of the same functional module (1) exhibit high congruence in genome-wide SFL interaction profiles (using an arbitrary congruence score cutoff ≥ 10) and/or in other mutant phenotypes such as sensitivity to exogenous DNA-damaging treatments (Tables S2–S5) and (2) do not exhibit direct SFL interaction with one another (between null alleles). The first criterion was used to functionally connect these components and the second to further assign them into the same or distinct modules depending on whether there is a direct SFL interaction between them.

With these criteria, we defined 16 functionally distinct modules important for DNA metabolism (Table 1; Figure S1). For simplicity, we named each module after a key component followed by an underlined m to indicate module except for modules with well-established names such as PPR (postreplication repair), HR (homologous recombination), and HIR, etc. (Table 1; Figure S1). We note that components of some modules were not used as queries in dSLAM screens and thus a genome-wide SFL profile was not available for each of them. They were included because of their physical interactions, shared SFL interaction partners, and more importantly, the lack of direct SFL interactions with other members of the same modules. The composition of the modules is highly consistent with results obtained from traditional genetic, biochemical, and physical interaction studies. For example, all 16 modules are either completely or partially in accord with known protein interaction studies done in yeast (Yeast Protein Database; Table 1; Figure S1).

Within all modules, highly similar SFL profiles were identified for all components and some were almost identical. A good example is the *RMD7*/*MDM39* module (*RMD7*m), where a link between *RMD7* and *MDM39* was not previously established. Here, we found almost identical SFL profiles for *rmd7*Δ and *mdm39*Δ (Figure 1A), and an *rmd7*Δ *mdm39*Δ double mutant grew no slower than either single mutant (data not shown). Some of the synthetic fitness defect (SF) interactions with *rmd7*Δ and *mdm39*Δ queries identified genes important for DNA replication and DNA repair (Figures 1B, 5A, and 5B). We thus predicted that *RMD7* and *MDM39* together define a biological function that contributes to DNA integrity. In support of this, the *rmd7*Δ and *mdm39*Δ mutants were similarly sensitive to methylmethane sulfonate (MMS) and hydroxyurea (HU; Figures 1C and 1D) but similarly insensitive to camptothecin (Cpt) and UV irradiation (data not shown). Consistent with the idea that *RMD7* and *MDM39* constitute a module rich in functional significance, both Rmd7p and Mdm39p were also previously shown to be required for mitochondrial morphogenesis and for meiosis in diploid yeast (Dimmer et al., 2002; Enyenihi and Saunders, 2003), to coexist in a multicomponent protein complex, and to colocalize in the endoplasmic reticulum (ER; Ho et al., 2002; Huh et al., 2003). This definition of *RMD7*m is consistent with a recent study defining it as part of the GET complex (Schuldiner et al., 2005).

RAD6/*BRE1*/*LGE1* (*BRE1*m) is another relatively new module involved in DNA metabolism. The three encoded proteins form a complex required for histone H2B ubiquitylation (Hwang et al., 2003; Wood et al., 2003). Both *RAD6* and *BRE1* were recently implicated in DNA-damage checkpoint signaling (Giannattasio et al., 2005), but a similar function was not established for *LGE1*. Here, we identified highly similar SFL profiles for these three genes (Table 1; Figure S1). In particular, SFL profiles of *bre1*Δ and *lge1*Δ (Figure 1A) and the DNA damage sensitivity of the *bre1*Δ and *lge1*Δ mutants (Figures 1C and 1D) were almost identical. The high congruence of the *bre1*Δ and *lge1*Δ phenotypes indicates that *LGE1* has a function similar to *BRE1* in DNA metabolism. Interestingly, *RMD7*m and *BRE1*m are functionally related: not only are all double mutants between these two modules lethal, a very specific subset of SFL interactions was also observed between both modules and multiple genes involved in chromatin remodeling (the *SWR1* complex) and histone modification (the Sin3p/Rpd3p histone deacetylase; Figure 1B). This suggests that like *BRE1*m, *RMD7*m may also regulate chromatin structure.

In some cases, however, components of the same module had truly distinct SFL partnerships. This is mainly because some components also function in other modules.

Table 1. Functionally Distinct Modules

Name of Module	Components of Module[a]	Congruency[b] of SL Profiles	SFL within Module[c]	Protein-Protein Interaction[d]
*BRE1*m	*RAD6*, *BRE1*, *LGE1*	22–109	No	Yes
CAF-I	*CAC2*, *MSI1*, *RLF2*	33–34	No	Yes
*CCR4*m	*CCR4*, *POP2*	133	No	Yes
*CSM1*m	*CSM1*, *LRS4*	65	No	Yes
*CTF18*m	*CTF18*, *CTF8*, *DCC1*	129–144	No	Yes
*HEX3*m	*HEX3*, *SLX8*	26	No	Yes
HIR	*ASF1*, *HIR1*, *HIR2*, ***HIR3***, ***HPC2***	13–40	No	Yes
HR	*RAD50*, *MRE11*, *XRS2*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*	55–116	No	Yes (only among Rad50p, Mre11p, and Xrs2p)
*MEC1*m	*MEC1*, ***LCD1***, *RAD53*	10	No	Yes (only between Mec1p and Lcd1p)
*MMS22*m	*MMS22*, *MMS1*, *RTT101*, *RTT107*	20–28	Yes[f] (only between *RTT101* and *RTT107*)	Yes (only among Mms22p, Rtt101p, and Rtt107p)
*MUS81*m	***MMS4***, *MUS81*	6[e]	No	Yes
*NAT1*m	*NAT1*, *ARD1*	184	No	Yes
PRR	*RAD6*, *RAD5*, *RAD18*	19–50	No	Yes
*RAD9*m	*RAD9*, *DDC1*, *RAD17*, *MEC3*, *RAD24*	27–38	No	Yes (only among Ddc1p, Rad17p, and Mec3p)
*RMD7*m	*RMD7*, *MDM39*	187	No	Yes
*TOF1*m	*TOF1*, *CSM3*	78	No	Yes

A graphical view of these modules is included as Figure S1.

[a] All genes except for those in boldface were used as queries in dSLAM screens.

[b] Congruency was calculated as described in Ye et al. (2005). Within a module, congruence scores shown represent the functional similarity between any two genes used as queries in dSLAM screens. For modules with more than two queries, a congruence score range was shown. In this study, a score of $\geq$10 was normally required for assigning two proteins into the same module.

[c] All possible pairwise SFL interactions were individually tested within a module, and the results were either Yes (positive) or No (negative).

[d] Protein-protein interaction information was obtained from the yeast proteome database (YPD). A positive (Yes) protein-protein interaction serves as further evidence that two congruent proteins belong to the same module.

[e] *MUS81* but not *MMS4* was used as a query, and the *MMS4* SFL interaction list likely was less complete. Thus with a congruence score at 6, these two were assigned into a module because there was physical interaction but no SFL interaction between the two components.

[f] Rtt101p and Rtt107p both bind to Mms22p; *RTT101* and *RTT107* were thus tentatively assigned to the same *MMS22* module despite their direct SF interaction. By a strict definition, this is not one module but two modules; among these two modules all the members are identical except that one contains Rtt101p and one contains Rtt107p. A possible interpretation of these facts is that Rtt101p and Rtt107p reside in mutually exclusive subcomplexes with Mms22p.

In addition to *BRE1*m, *RAD6* is also a component of a *RAD6*/*RAD5*/*RAD18*-dependent DNA postreplication repair (PRR) pathway. *rad6*Δ, *rad5*Δ, and *rad18*Δ shared a large number of SFL partners, particularly those directly involved in DNA metabolism (Figure 1B; Table S1). All three mutants were extremely sensitive to exogenous DNA-damaging treatments, more so than the *bre1*Δ and *lge1*Δ mutants (data not shown). However, unlike *RAD6*, an SFL interaction was not detected between the *RAD5* or *RAD18* and genes involved in chromatin remodeling and histone modification. Thus, truly distinct SFL interactions within a module likely indicate multiple functions for some of its components. This also suggests that the modules rather than the individual genes in many cases more accurately identify the basic functional units in a biological system.

Occasionally, SFL interactions between null mutants *within* a well-studied pathway were also observed, in apparent contradiction to our earlier assertion that such

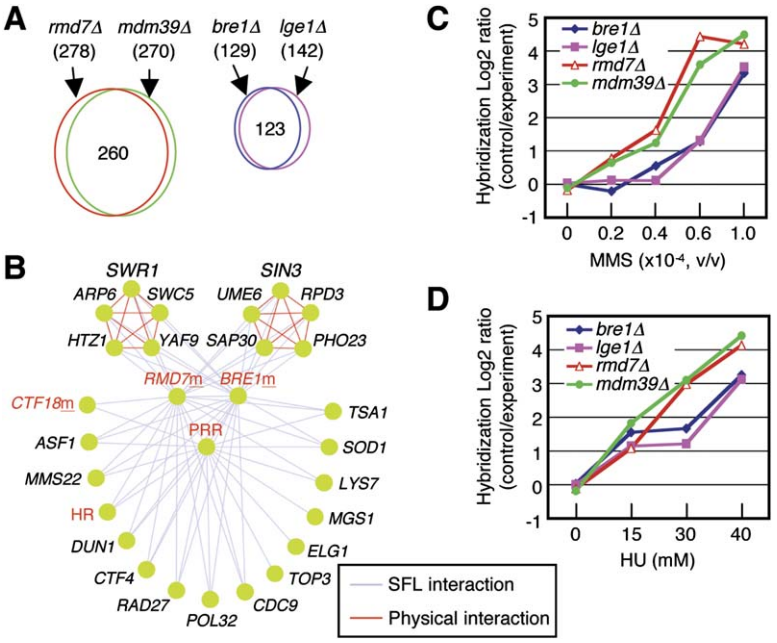

Figure 1. Novel Functional Modules Identified by Global Synthetic Lethality Analysis

(A) Highly similar SFL profiles between *rmd7*Δ and *mdm39*Δ and between *bre1*Δ and *lge1*Δ. In the parenthesis below each mutation is the number of SFL interactions identified. The number of interactions shared by *rmd7*Δ and *mdm39*Δ or by *bre1*Δ and *lge1*Δ is indicated within the ovals.

(B) SFL interactions among the *RMD7*m, *BRE1*m, and PRR modules and their interactions with DNA metabolism, histone modification (the Sin3p/Rpd3p histone deacetylase), and chromatin remodeling (the *SWR1* complex). This and all other network diagrams were created with network visualizing software Cytoscape 2.0 (Shannon et al., 2003). In all figures, the light green nodes represent individual genes (black fonts) or functional modules (red fonts), whereas the edges connecting the nodes represent SFL (light purple lines) or protein-protein (red lines) interactions. For clarity of the figures, only a subset of SFL interactions involving a given gene or module were displayed unless noted otherwise. Full interaction lists for each gene are presented in Table S1.

(C and D) MMS and HU sensitivity of the *rmd7*Δ, *mdm39*Δ, *bre1*Δ, and *lge1*Δ YKOs. Data for these two panels were derived from Tables S2 and S3. Sensitivity of each YKO was scored by microarray analysis of hybridization intensities of the corresponding TAGs in untreated (control) and treated (experiment) pools. A high Log2 ratio of control/experiment indicates that the YKO is sensitive to the genotoxic agent of indicated dosage.

interactions are not seen within a linear pathway. For example, the checkpoint kinase Dun1p acts partly downstream to the Rad9p/Ddc1p/Mec3p/Rad17p/Rad24p (*RAD9*m) of the DNA-damage checkpoint (DDC) pathway (Figure 2A), and yet SFL interactions were observed between *DUN1* and all five members of *RAD9*m (Figure 2B). We note that these genetic interactions actually involve a well-known *bifurcated* pathway: Dun1p is activated by Rad53p, which acts downstream to both the *RAD9*m and an S phase-specific DNA-replication checkpoint (DRC) pathway, whereas *RAD9*m activates both Dun1p and another checkpoint kinase Chk1p (Elledge, 1996; Rouse and Jackson, 2002; Figure 2A). The genetic interactions between *DUN1* and *RAD9*m can be explained by the SFL interactions between *RAD9*m and upstream components of the DRC pathway (*MRC1*, *TOF1/CSM3*, and *CTF18/CTF8/DCC1*, see below; Alcasabas et al., 2001; Foss, 2001; Tong et al., 2004) and that between *DUN1* and *CHK1* (Figures 2B and S2). Thus, SFL interactions can exist between null mutations within a *branched* pathway, and this does not contradict our previous simple model and assumption that SFL interactions do not occur between null mutations in the same *linear* pathway. In fact, our data provide a robust confirmation of the existing biochemical and genetic studies on these interconnected pathways.

CTF18/CTF8/DCC1 (*CTF18*m) and DNA-replication checkpoint

Almost identical SFL interactions were identified for all five components of *RAD9*m (Figure 2B). In addition to *DUN1*, *MRC1*, and *TOF1/CSM3*, other common SFL interactions include genes involved in DNA replication (*ELG1*, *RAD27*, *POL32*, and *CDC9*; the latter an essential gene revealed by hypomorphic mutation *ydl162c*Δ, see below), oxidative stress response (*SOD1* and *TSA1*), and more interestingly, all three members of a *CTF18/CTF8/DCC1* module (*CTF18*m) required for establishment of sister chromatid cohesion during DNA replication (Mayer et al., 2001; Figure 2B). Ctf18p and Rad24p are homologs and a role for Ctf18p in the S phase checkpoint was previously proposed (Naiki et al., 2001). The SF interactions between *CTF18* and all five members of *RAD9*m strongly indicate that Ctf18p does not substitute for Rad24p in checkpoint signaling but functions in parallel to the entire *RAD9* module (Figure 2A).

These genetic interactions also led us to predict a role of *CTF8* and *DCC1* of *CTF18*m in DNA-replication checkpoint signaling. To prove this, we first examined whether deletion of *CTF8* and *DCC1* causes defects in Rad53p phosphorylation in a *rad9*Δ mutant in response to exogenous DNA replication-blocking agents MMS and HU. Previous studies have shown that either of the DRC and DDC pathways is dispensable for Rad53p phosphorylation caused by blocked DNA replication, but mutations in both pathways abolish this response (Alcasabas et al., 2001; Foss, 2001; Tong et al., 2004). In support of our prediction, the *sml1*Δ *ctf8*Δ *rad9*Δ and *sml1*Δ *dcc1*Δ *rad9*Δ triple mutants but not the *sml1*Δ *ctf8*Δ, *sml1*Δ *dcc1*Δ, and *sml1*Δ *rad9*Δ double mutants are defective in Rad53p phosphorylation in response to MMS and HU

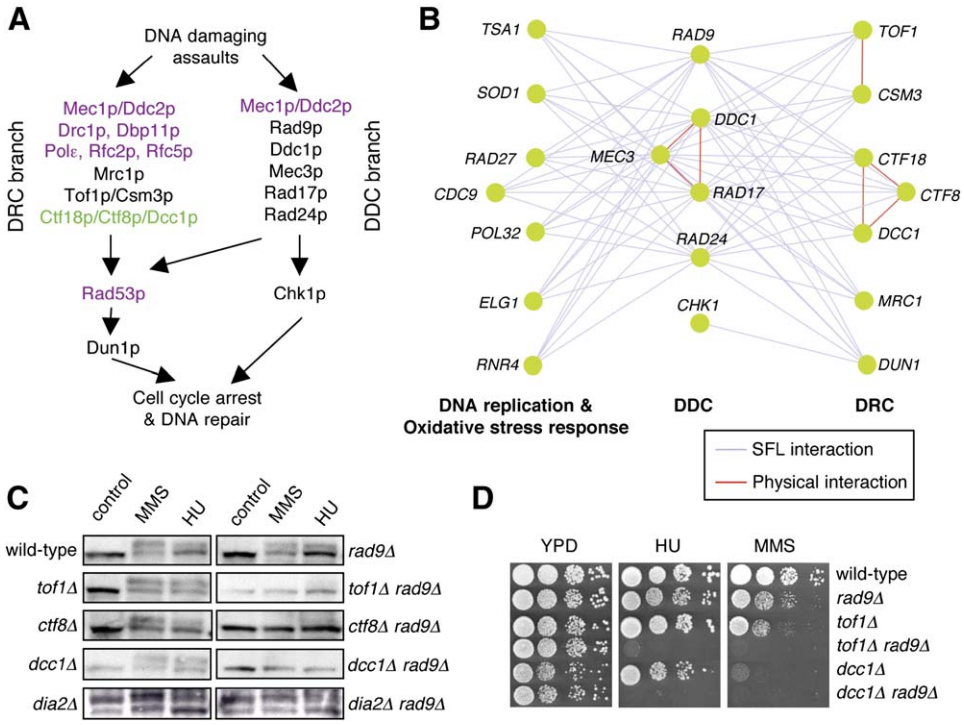

Figure 2. *CTF18*m Is Important for DNA-Replication Checkpoint Signaling

(A) A model of the DNA-replication and -damage checkpoint signaling in yeast. "DRC" stands for DNA-replication checkpoint and "DDC" for DNA-damage checkpoint. Signaling components colored in purple are essential for viability in wild-type yeast. Green color indicates that Ctf18p/Ctf8p/Dcc1p, as a module, is a novel component of the DRC pathway. Arrows represent the flow of biological information.

(B) A common set of SFL interactions identified for all five components of the *RAD9*m module of the DDC pathway.

(C) A role of *CTF8* and *DCC1* in Rad53p phosphorylation in response to exogenous DNA-damaging agents. To suppress potential growth defects of the *rad9*Δ-containing double mutants, an *sml1*Δ mutation was introduced into all strains shown in this and next panel. Extracts were prepared from asynchronous cells of the indicated genotypes grown in the presence or absence of MMS (0.05%) or HU (100 mM) and fractionated with 8% SDS-PAGE. An anti-Rad53p antibody was used to detect Rad53p; slow-migrating bands indicate Rad53p phosphorylation.

(D) Synthetic hypersensitivity to DNA damage conferred by *rad9*Δ and *dcc1*Δ mutations. 10× serial dilutions of log-phase cells of indicated genotypes were spotted on YPD medium that contained or lacked 50 mM HU or 0.01% MMS and incubated at 30°C for 3 (HU) or 2 days (MMS).

treatments (Figure 2C). Here, an *sml1*Δ mutation was used to suppress the slow growth phenotypes of the *ctf8*Δ *rad9*Δ and *dcc1*Δ *rad9*Δ mutants. In addition, the *sml1*Δ *dcc1*Δ *rad9*Δ triple mutant was more sensitive than the *sml1*Δ *dcc1*Δ and *sml1*Δ *rad9*Δ double mutants to lower concentrations of MMS and HU (Figure 2D). These effects of *CTF8* and *DCC1* on Rad53p phosphorylation were similar to that of *TOF1*, a DRC-signaling component (Figure 2C). Thus, *CTF18*m as a module is a new component of the DRC pathway (Figure 2A).

SFL interactions were also observed among *MRC1*, *TOF1*m, and *CTF18*m (Figure 3B), suggesting that they might define three different branches of the DRC pathway. Alternatively, these SFL interactions may reflect their functional redundancy in processes other than DRC signaling. Indeed, all three were previously implicated in DNA replication, sister chromatid cohesion, or DNA repair (Katou et al., 2003; Mayer et al., 2004; Osborn and Elledge, 2003; Warren et al., 2004; Xu et al., 2004). In further support of the later possibility, the *mrc1*Δ *csm3*Δ and *mrc1*Δ *tof1*Δ double mutants were not more defective than the *mrc1*Δ, *csm3*Δ, and *tof1*Δ single mutants in Rad53p phosphorylation when treated with MMS and HU (data not shown). Moreover, extensive Rad53p phosphorylation was observed in the double but not single mutants under unperturbed conditions (data not shown), indicating increased DNA damage, possibly due to more severe DNA replication defects in the double mutants.

The Sources of Spontaneous DNA Damage

Spontaneous DNA damage occurs under normal physiological conditions, and this leads to genomic instability. In agreement with previous studies (Bennett et al., 2001; Birrell et al., 2001; Parsons et al., 2004), we found in a systematic TAG array-based evaluation that mutants of the nucleotide excision repair (NER; *RAD1*/*RAD2*/*RAD10*/*RAD14*), the PRR, and the homologous recombination (HR; *RAD50*/*MRE11*/*XRS2*/*RAD51*/*RAD52*/*RAD54*/*RAD55*/*RAD57*/*RAD59*) repair pathways were extremely sensitive to treatments with UV, MMS, and HU, respectively (Figure S3). Thus, global synthetic lethality analyses with these pathways could reveal biological processes, mutation of

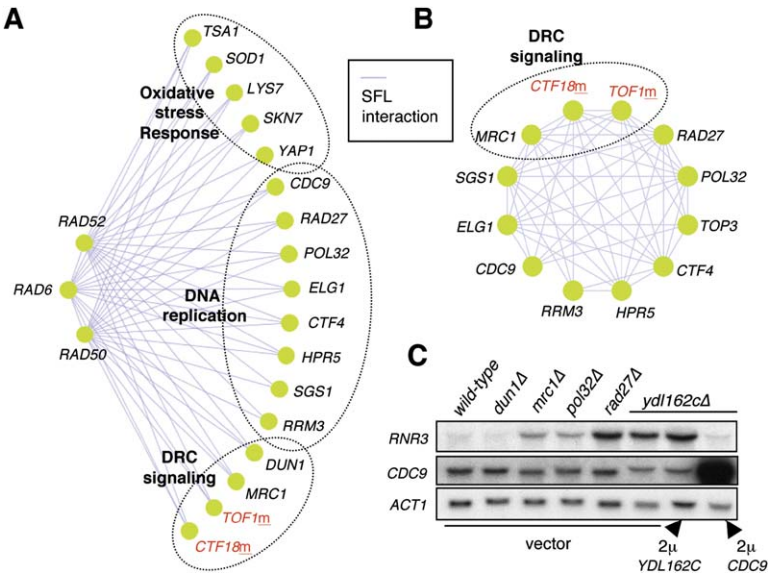

Figure 3. DNA Replication, DRC, and Oxidative Stress Response

(A) A subset of HR (*RAD50* and *RAD52*) and *RAD6* SFL interactions define genes required for DNA replication, DRC, and oxidative stress response.

(B) Enriched SFL interactions among components involved in DNA replication and DRC signaling.

(C) Increased *RNR3* expression in some DNA replication and DRC mutants under unperturbed conditions. Total RNA was isolated from log-phase culture of an isogenic wild-type (*ura3Δ::URA3*) strain, the *dun1Δ::URA3*, *pol32Δ::URA3*, *rad27Δ::URA3*, or *mrc1Δ::URA3* mutant carrying a vector plasmid (2μ, *LEU2*), or the *ydl162cΔ* mutant carrying a vector (2μ, *LEU2*), a *YDL162C* clone (2μ, *LEU2*), or a *CDC9* clone (2μ, *LEU2*) grown in SC-Ura-Leu medium in the absence of genotoxic stress and analyzed for *CDC9* and *RNR3* expression. *ACT1* expression was used as a loading control.

which causes elevated endogenous DNA damage under unperturbed conditions and thus to some extent mimics the exogenous DNA-damaging treatments.

Global synthetic lethality analyses with *rad2Δ* of the NER pathway did not identify any secondary mutation that conferred growth defects (data not shown). This is likely because none of the single mutations tested mimicked the effects of UV irradiation. In contrast, a large number of mutations impaired growth of mutants in the PRR and HR pathways. Among others, these included five genes required for oxidative stress response (*TSA1*, *SOD1*, *LYS7*, *SKN7*, and *YAP1*) and most of the testable known DNA replication and replication checkpoint components (*RAD27*, *POL32*, *RRM3*, *ELG1*, *CTF4*, *SGS1*, *HPR5*, *DUN1*, *MRC1*, *TOF1*m, and *CTF18*m) (Figure 3A). Some of these interactions were also previously reported (Huang and Kolodner, 2005; Tong et al., 2004). Although some of the replication genes are directly required for DNA repair (Figure 5A), which may underlie their SFL interactions with the PRR and HR pathways, additional evidence indicate that errors in DNA replication in general lead to endogenous DNA damage in the form of collapsed replication forks and/or double-strand breaks. As compared to an isogenic wild-type strain, increased *RNR3* expression, which reflects activation of checkpoint signaling by DNA damage (Huang et al., 1998), was observed in the *mrc1Δ*, *pol32Δ*, *rad27Δ*, and *ydl162cΔ* mutants under unperturbed conditions (Figure 3C). In addition, accumulation of single-stranded DNA fragments has been observed in *pol30* and *rad27Δ* mutants (Merrill and Holm, 1998). Consistent with this, we determined that *ydl162cΔ* actually represents a promoter-defective allele of the essential DNA ligase gene *CDC9*. The "gene" *YDL162C* was annotated as a "dubious ORF" with no known function (*Saccharomyces* genome database; http://www.yeastgenome.org/). This short ORF lies 105 bp upstream of the *CDC9* ORF, and its deletion reduced *CDC9* expression by ~50% in an asynchronous culture. A high-copy plasmid expressing *CDC9* but not the *YDL162C* ORF restored *RNR3* expression in the *ydl162cΔ* mutant to wild-type levels (Figure 3C). This phenomenon shows that, when studying the YKO collection mutants, one must be aware of the possibility that ORF deletions might compromise adjacent gene functions. We refer to this as an "off-by-one" error (Supplemental Data).

In the previous section, we discussed that, in addition to those of the DRC parallel pathway, genome-wide SFL interactions of *RAD9*m mainly involved genes important for oxidative stress response and DNA replication (Figure 2B). These results collectively suggest that DNA oxidation and errors in DNA replication are the major if not the only sources of potentially lethal endogenous DNA damage in yeast. Highly enriched SFL interactions were also observed among components required for DNA replication and DRC signaling (Figure 3B), suggesting that they have additive effects in preventing replication-specific DNA damage and thus belong to functionally distinct modules. These conclusions are consistent with and help explain previous observations of increased gross chromosomal rearrangement in multiple mutants defective in DNA replication, DRC signaling, and oxidative stress response (Huang et al., 2003; Kolodner et al., 2002; Lemoine et al., 2005). Presumably, increased spontaneous DNA damage promotes illegitimate DNA repair in these mutants.

Novel Components Implicated in DNA Replication

Our global SFL analysis likely has revealed additional genes functionally related to DNA replication. These include *DIA2*, *NPT1*, *HST3*, *HST4*, and the *CSM1/LRS4* module (*CSM1*m; Figure 4A).

1. DIA2

Dia2p is an F box protein that physically binds to the Skp1p-Cdc53p-Hrt1p SCF ubiquitin ligase and was

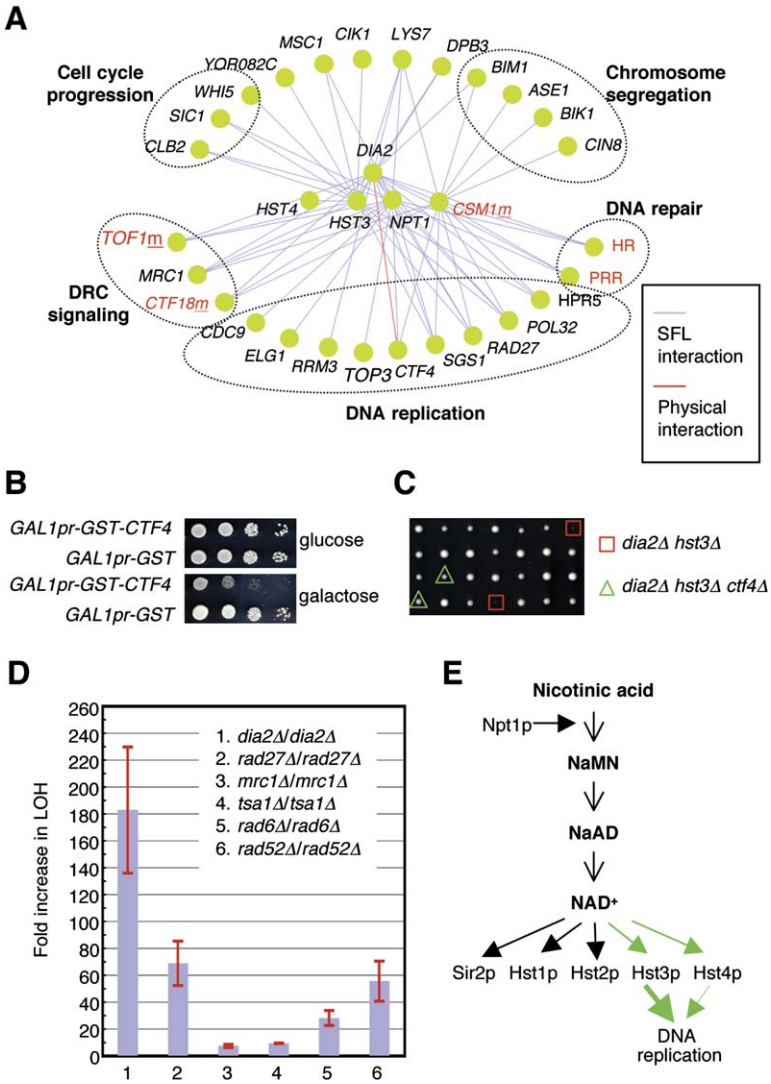

Figure 4. *DIA2*, *NPT1*, *HST3*, *HST4*, and *CSM1*m Are Functionally Linked to DNA Replication

(A) A subset of SFL interactions involving *DIA2*, *NPT1*, *HST3*, *HST4*, and *CSM1*m. All SFL interactions identified for *HST3* and *HST4* were shown.

(B) Overexpression of *CTF4* is toxic in yeast. The wild-type yeast BY4741 carrying a *GAL1pr-GST-CTF4* plasmid (URA3) or a control plasmid was grown in synthetic medium lacking uracil but containing either 2% glucose or 2% galactose as the sole carbon source. Cells were incubated at 30°C for 2 (glucose) or 3 (galactose) days.

(C) *ctf4*Δ suppresses lethality of the *dia2*Δ *hst3*Δ double mutants. Heterozygous diploid *DIA2/dia2*Δ*::natMX HST3/hst3*Δ*::kanMX CTF4/ctf4*Δ*::URA3* was sporulated and dissected on YPD plate. Genotypes of each haploid progeny from this dissection were determined by growth on YPD plus CloNat (*dia2*Δ) or G418 (*hst3*Δ) or on synthetic medium lacking uracil (*ctf4*Δ).

(D) *DIA2* is required for suppressing LOH at the mating-type locus. Fold increases in LOH frequency of homozygous diploid strains were compared to isogenic wild-type strains: *dia2*Δ/*dia2*Δ (183 ± 47 fold); *rad27*Δ/*rad27*Δ (69.0 ± 16.4 fold); *mrc1*Δ/*mrc1*Δ (7.47 ± 1.29 fold); *tsa1*Δ/*tsa1*Δ (9.41 ± 0.33 fold); *rad6*Δ/*rad6*Δ (28.2 ± 5.46 fold); *rad52*Δ/*rad52*Δ (55.6 ± 14.8 fold). Two independent isolates of each genotype were tested, and the results were averaged.

(E) Npt1p, NAD^+, Hst3p, and Hst4p define a pathway contributing to DNA replication. Heavy arrows indicate flow of biological information, whereas thin arrows symbolize metabolic conversions. Green arrows represent new conclusions inferred from this study.

recently shown to be required for ubiquitin-dependent protein degradation (Bao et al., 2004; Kus et al., 2004). In addition to the DNA-repair pathways, SFL interactions were observed between *dia2*Δ and mutations of most testable DNA replication and DRC signaling genes (Figure 4A), suggesting that Dia2p itself might regulate a DNA replication-related process. One interesting exception to this trend among the known replication genes was *CTF4*; Ctf4p was previously found to physically bind to Dia2p (Ho et al., 2002; Figure 4A). In addition to an absence of interaction by dSLAM, no SFL interactions were seen between *ctf4*Δ and *dia2*Δ by random spore and tetrad analyses, suggesting that Dia2p and Ctf4p function in the same pathway. However, the *ctf4*Δ mutants are highly sensitive to DNA-damaging treatments (MMS, HU, UV, and Cpt), whereas the *dia2*Δ mutants were only modestly sensitive to MMS (Tables S2–S5). These results, together with Dia2p's role in ubiquitylation, suggest that Dia2p might regulate turnover of Ctf4p, aberrant accumulation of which is detrimental to DNA integrity. Indeed, overexpression of a GST-Ctf4p fusion protein was toxic in yeast (Figure 4B). Alternatively, the deleterious effects of Ctf4p might be inhibited by ubiquitylation or direct binding by Dia2p. Consistent with these models, a *ctf4*Δ mutation suppressed certain phenotypes of a *dia2*Δ mutation. While *dia2*Δ *hst3*Δ double mutants were extremely slow growing, the *ctf4*Δ *dia2*Δ *hst3*Δ triple mutants grew similarly to a *ctf4*Δ single mutant (Figure 4C). In contrast, deletion of the known Dia2p substrate Tec1p (Bao et al., 2004) did not restore robust growth to *dia2*Δ *hst3*Δ mutants (data not shown).

We also directly investigated the effect of *dia2*Δ on genomic stability. As compared to isogenic wild-type strains, the frequency of loss of heterozygosity (LOH) at the mating-type locus in *dia2*Δ/*dia2*Δ diploid mutants was increased by an average of >100-fold (Figure 4D). This effect of *dia2*Δ on LOH was more prominent than those caused by mutations of the major DNA-repair pathways (*rad52*Δ

and *rad6*Δ) and oxidative stress response (*tsa1*Δ; Figure 4D). In addition, we also found extensive Rad53p phosphorylation in a *dia2*Δ mutant under unperturbed conditions that was not abolished by a *rad9*Δ mutation, suggesting activation of the DRC pathway (Figure 2C). These observations are consistent with the idea that the *dia2*Δ mutants have defects in DNA replication, which lead to spontaneous DNA damage and genomic instability.

2. NPT1, HST3, and HST4

Npt1p is a nicotinate phosphoribosyltransferase required for biosynthesis of NAD^+ via the salvage pathway in yeast (Rajavel et al., 1998; Figure 4C). SFL interactions were observed between *npt1*Δ and the major DNA-repair pathways as well as multiple DNA replication and DRC components (Figure 4A). These results suggest that Npt1p or the level of NAD^+ produced regulates DNA replication. This idea was further supported by studies of Hst3p and Hst4p, two of the four Sir2p homologs in yeast (Brachmann et al., 1995) which, like Sir2p, likely use NAD^+ as a cofactor. A global SFL screen with *hst3*Δ identified 18 interactors (Tong et al., 2004 and this work), most of which affected DNA replication (*RAD27*, *POL32*, *SGS1*, *HPR5*, and *DIA2*), DRC (*MRC1* and *TOF1*m), and entry into S phase of the cell cycle (*SIC1* and *WHI5*; Amon, 1998; Costanzo et al., 2004; de Bruin et al., 2004; Figure 4A). A similar screen with *hst4*Δ identified only *hst3*Δ (Figure 4A). Hst3p and Hst4p are homologs (34% identity); this genetic interaction thus suggests that they together define an activity and Hst3p plays a more prominent replication-related role. Interestingly, Hst3p physically binds to the origin of DNA replication on a 2μ plasmid (Grunweller and Ehrenhofer-Murray, 2002), and the growth defect of an *hst3*Δ *hst4*Δ double mutant was partially suppressed by overexpression of the largest subunit of the replication factor C, Rfc1p (I. Celic and J.D.B., unpublished data). These results together strongly indicate that *HST3* and *HST4* are involved in DNA replication.

*npt1*Δ shared most of the *hst3*Δ interactions (Figure 4A), and no direct SFL interaction was observed between them (data not shown), indicating action in the same pathway (Figure 4E). In addition, NAD^+ is a metabolic product of Npt1p, and it serves as a cofactor for Sir2p and two other Sir2p homologs: Hstp1 and Hst2p (Imai et al., 2000; Landry et al., 2000; Sutton et al., 2001). Although it is not known whether Hst3p and Hst4p activity similarly requires NAD^+, our genetic data on *NPT1*, *HST3*, and *HST4* suggest that NAD^+ could serve as a cofactor for Hst3p and Hst4p. Thus, Npt1p, NAD^+, Hst3p, and Hst4p likely define a NAD^+-dependent pathway important for DNA replication in yeast (Figure 4E).

3. CSM1/LRS4

The gene products of *CSM1* and *LRS4* are components of the monopolin complex required for monopolar attachment of homologous chromosomes to the meiotic spindle in meiosis I (Rabitsch et al., 2003) and results of global SFL analyses suggest that the two define a functional module (*CSM1*m; Table 1; Figure S1). Synthetic genetic interactions were observed between *CSM1*m and multiple genes critical for mitotic chromosome segregation (*ASE1*, *BIK1*, *BIM1*, and *CIN8*), indicating that *CSM1*m is also important for chromosome dynamics during mitosis in budding yeast (Figure 4A). Interestingly, SFL interactions were also observed between *CSM1*m and some major DNA-repair pathways (PRR and HR) as well as genes directly involved in DNA replication (Figure 4A). Moreover, Csm1p was previously shown to physically bind to DNA replication proteins Mcm3p, Mcm5p, Mcm7p, and Clf1p (Wysocka et al., 2004). These together suggest that *CSM1*m also plays a mitotic role in DNA replication.

Multiple Pathways Required for Repairing Endogenous DNA Damage

In addition to their genetic interactions with DNA replication and oxidative stress response, members of the PRR and HR pathways also exhibited SF but not SL interactions with each other (Figure 5), consistent with the idea that they act in parallel and in an important but not essential manner. The fact that double mutants of these two pathways retain viability indicates that additional pathways must exist to repair endogenous DNA damage. Mutants of such additional pathways likely are also sensitive to increased endogenous DNA damage caused by defects in DNA replication and oxidative stress response. Here, we discuss a common subset of such pathways or genes required for normal cell growth when either the DNA replication machinery (crippled by *rad27*Δ) or the oxidative stress response (diminished by *tsa1*Δ) was compromised (Table S6). Consistent with their roles in DNA repair, mutants of these genes were also sensitive to low doses of exogenous DNA-damaging treatments (Table S6). These include the DDC-signaling components (the *RAD9*m and *MEC1*m modules), some DNA-replication components (*CTF4*, *HPR5*, *POL32*, *SGS1*, and *CTF18*m), and others likely involved in DNA repair (Figure 5A). Most of these other DNA-repair genes exhibited SF interactions with either the HR or *RAD6*-dependent pathways or both under unperturbed conditions. They also exhibited mutual synthetic interactions (Figure 5B), suggesting that they define distinct DNA-repair pathways. Among these, the *MMS22*m module (*MMS22/MMS1/RTT101/RTT107*) is most likely required for DNA double-strand break repair because the corresponding mutants were all hypersensitive to MMS, HU, and camptothecin but not to UV irradiation (Tables S2–S5). Together with HR and the *RAD6*-dependent pathways, the additional pathways described here comprise a network of activities that protect against or repair the endogenous DNA damage created by oxidation or during DNA replication.

Chromatin structure maintenance has been shown to be important for survival of yeast cells exposed to exogenous genotoxic treatments (Morrison and Shen, 2005). Here, we also observed SFL interactions between multiple DNA-replication genes (*DIA2*, *RAD27*, and *POL32*) and genes of complexes or pathways involved in histone modification (*VID21/EAF3/EAF5/YAF9*; Krogan et al., 2004), chromatin remodeling (*ARP8/IES2* and *ARP6/SWC5/*

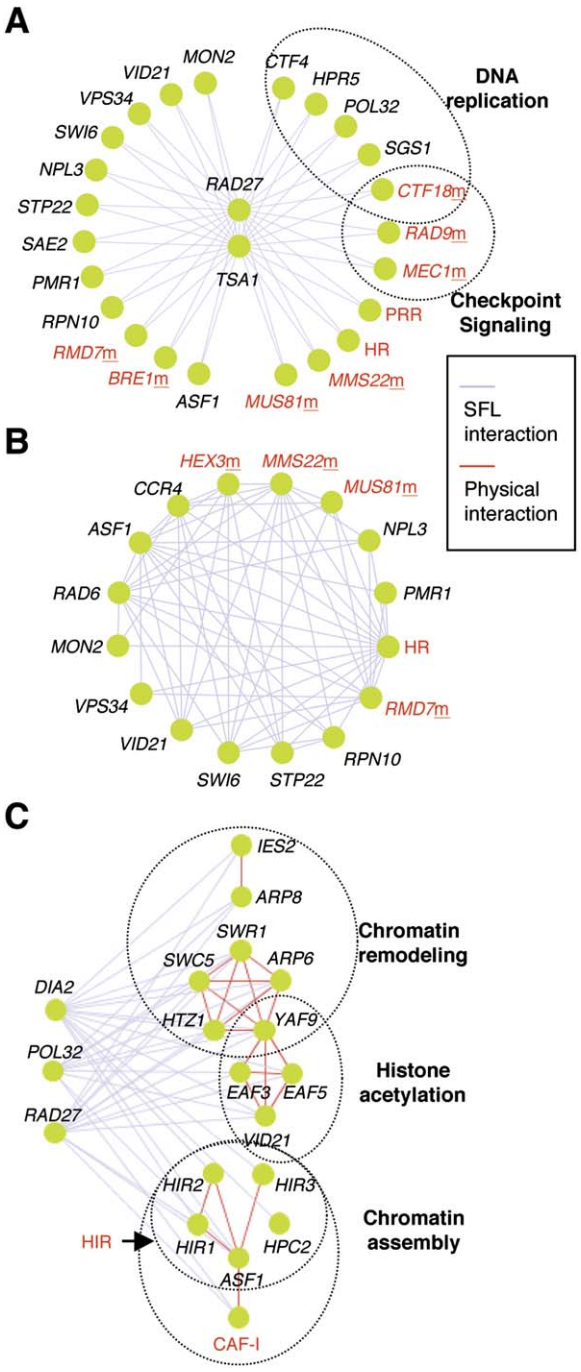

Figure 5. SFL Interactions Define Multiple Pathways for Repairing Endogenous DNA Damage

(A) A common set of genes or modules required for normal cell growth in the absence of either *RAD27* or *TSA1*.

(B) SFL interactions define distinct DNA-repair genes or modules.

(C) Genetic interactions between DNA replication and chromatin structure maintenance.

SWR1/*YAF9*/*HTZ1*; Shen et al., 2003), and chromatin assembly (the CAF-I and HIR modules; Sharp et al., 2001; Figure 5C). This provides strong evidence that chromatin-structure maintenance plays an important role in preventing and/or repairing endogenous DNA damage. Interestingly, we also observed extensive SFL interactions between these chromatin-structure maintenance genes and *RAD53* (as *rad53*Δ *sml1*Δ) but less so with *MEC1* (as *mec1*Δ *sml1*Δ) (Figure S4; Table S1), suggesting that *RAD53* might be directly involved in chromatin structure maintenance (see Supplemental Data).

DISCUSSION

SFL Modules

Here, we have identified a complex genetic network governing DNA integrity in *S. cerevisiae*. The portion of the network dissected here involves 4956 unique interactions built around 74 query genes. Most of these query genes can be organized into 16 functional modules (Table 1; Figure S1), indicating that global SFL analyses can be effectively exploited to dissect pathway topology in addition to identifying functionally connected gene pairs. This is because components of a linear pathway normally share similar SFL partners (Tong et al., 2004) but tend not to exhibit SFL interactions with one another, especially when null mutations are exclusively involved (Kelley and Ideker, 2005; Ye et al., 2005). A conceptually similar approach (with some important differences) to interpreting SFL interactions was recently exploited to dissect the early secretory pathway (Schuldiner et al., 2005). The modules defined in our study are highly consistent with known protein-protein interaction data and traditional genetic and biochemical analyses. The modularity functionally connects or unifies components of each module. These modules also serve as the basic building blocks for a more complex biological network governing genomic integrity in yeast. We note that our second criterion for defining modules should be applied cautiously when the genetic interactions involve partial loss-of-function alleles. In such cases, SL interactions could also arise from consecutive reduction of activities in the same pathway.

Significance of This Network

Defects in DNA replication and oxidative stress response have been shown to cause DNA damage and genomic instability (Huang and Kolodner, 2005; Myung et al., 2001). Our data from comprehensive genome-wide analyses suggest that DNA replication and oxidation are the major sources of endogenous DNA damage and genomic instability in yeast. Defects in the oxidative stress response, DNA replication, and DRC signaling allow for potentially lethal spontaneous DNA damage to occur (Figure 6A). The extensive SFL interactions between nearly every testable DNA-replication gene and the major DNA-repair pathways also allow the prediction that loss-of-function mutations in most if not all DNA-replication genes will promote genomic instability. Activation of the DDC- or DRC-signaling pathways by endogenous DNA damage or replication stress causes cell cycle arrest, which allows more

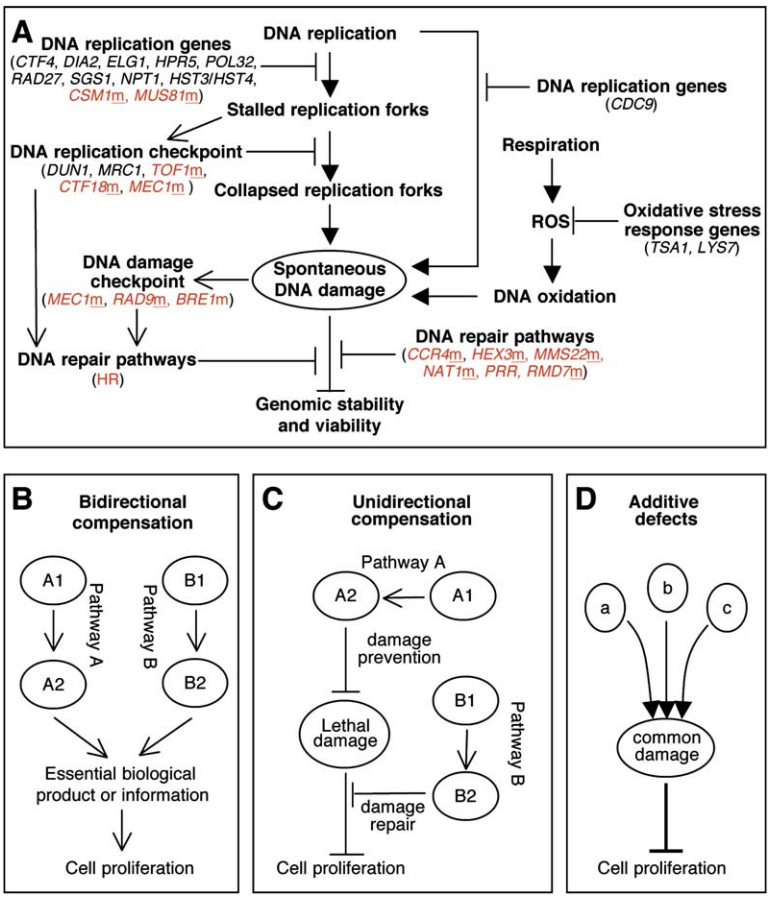

Figure 6. Underlying Mechanisms for Synthetic Lethality between Null Alleles

(A) A diagram of compensatory biological processes that collectively contributes to suppression of spontaneous DNA damage and genomic instability in yeast. Genes or modules were tentatively assigned to different pathways or processes according to GO annotations and mutant phenotypes. These assignments were oversimplified as some genes and modules have multiple functions and participate in multiple processes. "ROS" stands for reactive oxygen species. The arrows indicate flow information flow whereas the perpendiculars stand for inhibition of information flow.

(B) Bidirectional functional compensation between parallel pathways (A and B). Synthetically lethal pairs define components of two parallel pathways that have a common output essential for cell viability.

(C) Unidirectional compensation. Pathway A prevents the occurrence of potentially lethal damage, which is repaired by pathway B.

(D) Additive defects in multiple pathways (a, b, and c) of a common biological process lead to slowed cell proliferation or lethality.

time for DNA repair. In addition, these signaling pathways actively participate in the repair process by directly regulating DNA-repair pathways and activating expression of genes required for DNA repair (Zhou and Elledge, 2000). The different repair pathways compensate for defects in DNA replication and oxidative response by repairing DNA damage. They also compensate for one another (Figure 5B; Table S6) but likely have distinct roles because mutation of each pathway alone causes sensitivity to DNA damage. Together, they are essential for efficient repair of the endogenous DNA damage and cell survival. The lethality and slow-growth phenotypes of a significant portion of the double mutants discussed here likely have resulted from increased endogenous DNA lesions, which either kill the cells or delay cell cycle progression by activating the DNA-damage checkpoint. A number of pathways and genes discussed here were previously linked to increased gross chromosomal rearrangement (Huang et al., 2003; Kolodner et al., 2002). Thus, this genetic network will help identify mutations, either alone or in combination, that cause increased endogenous DNA damage and genomic instability in yeast. As an example, we found activation of the DNA-damage checkpoint signaling and elevated LOH rates when *DIA2* was deleted (Figures 2C and 4C).

Human homologs of some of the yeast DNA metabolism genes have been linked to cancer, aging, and neuronal degeneration (Kolodner et al., 2002). Activation of the DNA-damage checkpoint, which indicates increased spontaneous DNA damage, was also observed during early steps of human tumorigenesis (Bartkova et al., 2005; Gorgoulis et al., 2005). Susceptibility to human diseases and longevity are traits with a strong genetic component but are "complex". The human gene combinations responsible for these traits are of intense interest but difficult to identify. Because of the strong conservation of yeast and human "monogenic" disease genes (Bassett et al., 1997), it is likely that our genetic network will provide a basis for helping identify mutations that, in combination, contribute to the development of human diseases. Moreover, the SL interactions discovered in yeast could aid in selecting human homologs as targets for anticancer therapies. Drugs targeting the SL partners of a cancer-specific mutation could selectively kill cancer cells and be less toxic to normal tissues (Hartwell et al., 1997).

Multiple Paths to Synthetic Lethality

Synthetic lethality can occur between mutations within the same pathway if partial loss-of-function alleles are studied (Hartman et al., 2001). Yet the vast majority of SFL

interactions between null mutations, as studied here, reflect functional relationships between pathways (Kelley and Ideker, 2005; Ye et al., 2005). This happens, as in the case of DRC and DCC (Figure 2), between alleles of strictly parallel pathways with a common output (Figure 6B), with both pathways functionally compensating for each other in a *bidirectional* manner. SFL interactions sometimes also reflect *unidirectional* compensation. For example, the oxidative stress-response system prevents accumulation of DNA-damaging reactive oxygen species (ROS) but does not directly participate in DNA repair. Its failure leads to oxidative DNA damage, which requires efficient repair by a variety of DNA-repair pathways for cell survival. This was revealed by the SL interactions between mutations of oxidative stress response and those of both the PRR and HR pathways (Huang and Kolodner, 2005; this study; Figure 5). Here, the PRR and HR pathways compensate for the defects in the oxidative stress response but not the other way around. This kind of *unidirectional* compensation (Figure 6C) could also explain the SFL interactions observed between genes of DNA replication and those of DNA repair and checkpoint signaling. In other cases, SFL interactions arise from *additive defects* in multiple pathways that contribute to a common biological process, and these pathways may not compensate for one another (Figure 6D). For example, DNA replication is a complex process involving a multitude of coordinated subpathways or well-defined activities. Moreover, it is a process in which high fidelity is critical to viability. Extensive SFL interactions were observed among all testable (i.e., nonessential) DNA replication mutations, likely because of slowed S phase progression in the double mutants and/or their loss of viability as a result of extensive replication-related DNA damage, which can be viewed as surpassing a replication fidelity threshold. This "additive defects in a common high-fidelity process" model (Figure 6D) is distinct from the view that SL interactions arise from additive effects of any two "sick" mutations. The majority of SFL interactions reported so far are between functionally related genes (Tong et al., 2004; this study).

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids

Yeast strains used in this study were all derived from BY4741 (*MAT***a**) or BY4743 (*MAT***a**/α) (Brachmann et al., 1998); strains and plasmids are listed (Table S7).

dSLAM Screens

Global synthetic lethality screens, including preparation of the query constructs, were performed essentially as previously described (Pan et al., 2004) with subtle modifications. In most cases, the magic medium (MM; SC-Leu-His-Arg + canavanine + G418), rather than MM + 5-FOA, was used to select for a mixed population of haploid single (*xxx*Δ*::kanMX*) and double (*yfg*Δ*::URA3 xxx*Δ*::kanMX*) mutants (instead of the *xxx*Δ*::kanMX* single mutants only) as the control pool. For the *rad53*Δ *sml1*Δ and *mec1*Δ *sml1*Δ screens, an *sml1*Δ*::URA3* cassette was first transformed into the pool of haploid-convertible heterozygous diploid YKOs to create a heterozygous double-mutant pool, which was then transformed with either a *rad53*Δ*::natMX* or a *mec1*Δ*::natMX* cassette to create a triple-mutant pool. Synthetic lethality analysis was then carried out as for the *cdc102-1* allele (Pan et al., 2004). To minimize false negative rates, YKOs with a control/experiment ratio $\geq$2 with either UPTAG or DOWNTAG or with ratios $\geq$1.5 with both TAGs were selected for individual confirmation tests with random spore analysis or tetrad dissection. Primers for PCR amplification of the query constructs were listed in Table S8.

Confirmation of SFL Interactions

Transformation of the query constructs into the individual haploid-convertible heterozygous diploid YKOs of the target genes was carried out using a 96-well high throughput transformation protocol (X.P. and J.D.B., unpublished data). Two or more independent transformants for each were tested either by random spore analysis (RSA) or tetrad dissection on YPD or both. For most RSAs, haploid progenies were spotted as 10$\times$ serial dilutions on MM (selects for *xxx*Δ*::kanMX* and *yfg*Δ*::URA3 xxx*Δ*::kanMX* cells), MM-Ura-G418 (select for *yfg*Δ*::URA3* and *yfg*Δ*::URA3 xxx*Δ*::kanMX* cells), and MM-Ura (selects for only the *yfg*Δ*::URA3 xxx*Δ*::kanMX* double-mutant cells) and incubated at 30°C for 2 to 3 days. SFL interactions were scored by comparing the colony formation and colony sizes of the double mutant with those of the single mutants. RSA confirmation of the *rad53*Δ *sml1*Δ results was similarly carried out by comparing the growth on MM-Ura + CloNat versus on MM-Ura-CloNat and MM-Ura + CloNat-G418.

Other Techniques

For RNA blot analysis, cells from 25 ml log-phase cultures of synthetic medium lacking L-leucine and uracil (SC-Leu-Ura) were used to isolate total RNA, which was fractionated, blotted, and probed with *RNR3* (nt 2299 to 2527), *CDC9* (nt 1816 to 2247), and *ACT1* (nt 500 to 999) ORF DNA sequences. Rad53p phosphorylation was revealed by slower-migrating bands on 8% SDS-PAGE. This was performed as described (Alcasabas et al., 2001) by using an anti-Rad53p antibody (yc-19, Santa Cruz). To study effects of mutations on the frequency of LOH at the *MAT* locus, two isolates were independently constructed for the wild-type and each homozygous diploid mutant. These diploid strains all carry a *MAT***a**-specific reporter (*can1*Δ*::LEU2-MFA1pr-HIS3*; Pan et al., 2004). They do not express *HIS3*, which is controlled by the *MAT***a**-specific *MFA1* promoter because there is both **a** and α information at the *MAT* locus and thus they cannot grow on medium lacking histidine. Inactivation of *MAT*α information by mutation, genome rearrangement, or chromosome loss allows expression of *HIS3*. Cells of each genotype were spread on SC-Leu or SC-Leu-His. The number of Leu^+ His^+ colonies divided by that of total Leu^+ colonies (Leu^+ His^+ and Leu^+ His^-) of the same inoculum was used to calculate the LOH frequency of a strain.

Supplemental Data

Supplemental Data include five figures, eleven tables, and supplemental text and can be found with this article online at http://www.cell.com/cgi/content/full/124/5/1069/DC1/.

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